

## NON-ALLOYING CORE SHELL NANOPARTICLES

### **CROSS-REFERENCE:**

5        This application claims the benefit of priority from U.S. Provisional application No. 60/293,861, filed May 25, 2001, which is incorporated by reference in its entirety. The work reported in this application has been supported, in part, by NSF grant no. CHE-9871903; ARO grant no. DAAG55-97-1-0133, and AFOSR grant no. DURINT. Accordingly, the U.S. government may have some rights to the invention.

### **FIELD OF INVENTION:**

10        The present invention relates to core/shell nanoparticles, materials based on core/shell nanoparticles, kits containing core/shell nanoparticles, and methods of making and using core/shell nanoparticles for the detection of target molecules, including nucleic acids, peptides, and proteins. In particular, the present invention relates to specific binding substance-modified  
15        core/shell nanoparticles such as DNA-modified core/shell nanoparticles and their use for detecting target molecules such as nucleic acids.

### **BACKGROUND OF INVENTION:**

20        In 1996, a method was reported for utilizing biomolecules, such as DNA, and their molecular recognition properties to guide the assembly of nanoparticle building blocks modified with complementary recognition elements into functional materials.<sup>1</sup> These materials have found wide application in the development of highly sensitive and selective diagnostic methods for DNA.<sup>2</sup> This material synthesis approach has been extended to a wide range of  
25        biomolecules, including peptides and proteins,<sup>3</sup> and a modest collection of nanoparticles including gold and semiconductor quantum dots.<sup>4-9</sup> In each case, when a new nanoparticle composition is designed, new modification methods must be developed for immobilizing biomolecules on the surface of the particles of interest. This approach has been extensively utilized but with limited success. The methods for modifying gold nanoparticles have now  
30        been optimized and generalized for a wide range of particle sizes and surface compositions, including spheres and rods.<sup>1,2,4,10</sup> Gold particles are particularly easy to modify because they are often stabilized with a weakly binding layer of charged ligands (e.g. citrate) that can be replaced with molecules with chemical functionalities that bind more strongly (e.g. thiols, amines, and disulfides) to their surfaces than these ligands. The CdSe and CdS quantum dots  
35        have proven more difficult to modify because they have a surfactant layer that is very strongly

bound to their surfaces and, consequently, difficult to displace.<sup>5</sup> No successful routes have been developed for creating stable oligonucleotide conjugates with silver nanoparticles, primarily because they tend to chemically degrade under conditions used to effect DNA hybridization. A major advance would be to devise a method for designing particles with the physical properties of a chosen nanoparticle composition but the surface chemistry of gold. Herein, a low temperature method is provided for generating core/shell particles consisting of a silver core and a non-alloying gold shell that can be readily functionalized with oligonucleotides using the proven preparatory methods for pure gold particle oligonucleotide conjugates.<sup>2d</sup> Moreover, the novel nanoparticle composition can be used to access a colorimetric detection system distinct from the pure gold system.<sup>2a,2d</sup>

### **BRIEF SUMMARY OF THE INVENTION:**

The present invention relates to composite core/shell nanoparticles, compositions and kits including these core/shell nanoparticles, and methods for preparing and using composite core/shell nanoparticles, particularly Ag/gold core/shell nanoparticles, for the detection of target molecules such as nucleic acids, proteins and the like. These Ag/gold core/shell nanoparticles were prepared by reduction of  $\text{HAuCl}_4$  by  $\text{NaBH}_4$  in the presence of Ag-nanoparticle “templates” and characterized by UV-vis spectroscopy, transmission electron microscopy (TEM), and energy dispersive X-ray (EDX) microanalysis. Significantly, these particles do not alloy, yielding structures with the optical properties of silver and the surface chemistry and high stability of Au. Experimental and theoretical data support the structural characterization of these novel materials as silver cores (~12 nm in diameter) coated with approximately one atomic monolayer of gold (~3 Å). The core/shell nanoparticles may be further modified with alkanethiol-oligonucleotides forming structures that undergo reversible hybridization with complementary oligonucleotides to form extended nanoparticle network structures. By spotting aliquots of a solution containing the oligonucleotide-modified nanoparticles without and with DNA target on a reverse-phase alumina plate, a distinct colorimetric transition from yellow to dark brown can be observed by the naked eye. The optical properties of the dispersed and aggregated core/shell particles form a new colorimetric channel for nanoparticle based DNA detection.

Accordingly, one object of the invention is to provide straightforward method of preparing core/shell nanoparticles with the optical, and many of the physical, properties of silver but the stability of gold. The surfaces of these nanoparticles can be modified with a

variety of moieties such as, for example, natural and synthetic polymers, molecules capable of selective molecular recognition including, but not limited to, nucleotides, nucleosides, poly- or oligonucleotides, proteins, peptides, carbohydrates, sugars, and haptens, thereby providing useful biorecognition properties to the nanoparticles.

Another object of the invention is to provide a general method for preparing core/shell particles with tailorable physical properties by virtue of choice of core, e.g.,  $\text{Fe}_3\text{O}_4$ , Cu or Pt, but the surface chemistry and stability of the native, and oligonucleotide modified, pure gold particles.

Another object of the invention is to provide methods for detection of molecules capable of selective molecular recognition comprising use of core/shell nanoparticle probes. These methods comprise contacting the core/shell nanoparticle probes with one or a plurality of target molecules under conditions that allow for selective molecular recognition, and the detection of an optical change. The physical properties of the particular core/shell nanoparticle probes can allow for various additional steps in these methods such as, for example, inducing their migration through application of electrical or magnetic fields.

Another object of the invention is to provide nanomaterials based on the core/shell nanoparticles of the invention.

These and other objects of the invention will become apparent in light of the detailed description below.

#### **BRIEF DESCRIPTION OF THE DRAWINGS:**

Figure 1 illustrates (A) a TEM image of Ag/Au core/shell nanoparticles; (B) EDX spectra of silver core nanoparticles (dotted line) and Ag/Au core/shell nanoparticles (solid line) wherein L and M signify electron transitions into the L and M shell of the atoms, respectively, from higher states; (C) UV-vis spectra of silver core (dotted line) and Ag/gold core/shell (solid line) wherein the inset shows the calculated extinction spectra of silver nanoparticles (dotted line) and Ag/Au core/shell nanoparticles (solid line); (D) Thermal denaturization curve of aggregates formed from hybridized oligonucleotide modified Ag/Au core/shell nanoparticles in buffer solution (0.3 M NaCl and 10 mM phosphate buffer, pH=7). The inset shows the UV-vis spectra of dispersed oligonucleotide-modified Ag/Au core/shell nanoparticles (solid line) and aggregated (dotted line) oligonucleotide-modified Ag/Au core/shell nanoparticles formed via hybridization. The base sequences are given in Figure 2A.

Figure 2 illustrates (A) Mercaptoalkyl-oligonucleotide-modified Ag/Au core/shell particles and an oligonucleotide target. Represents the core/shell nanoparticle and “~” represents a propyl (left) or hexyl (right) group linking S to the oligonucleotide probe. DNA spot test using: (B) 12.4-nm Ag/gold nanoparticle probes and (C) 13-nm gold nanoparticle probes: (I) without target, (II) with target at room temperature, (III) with target at 58.0 °C, a temperature above the  $T_m$  (53.0 °C) of the hybridized DNA.

Figure 3 illustrates the UV-VIS spectra of a Pt core (dotted line) and Pt/gold core/shell nanoparticles (solid line).

Figure 4 illustrates the UV-VIS spectra of gold growth on the surface of  $\text{Fe}_3\text{O}_4$  nanoparticles at 0, 0.3 nm, 0.6 nm, and 0.9 nm thickness.

Figure 5 illustrates the behavior of  $\text{Fe}_3\text{O}_4$ /gold core/shell particles as super paramagnetic particles in the presence of an applied magnetic field. In the presence of a magnetic field, a solution containing the magnetic gold nanoparticles appears red. When a magnetic force is applied over a period of 2 hours, the solution becomes colorless as the nanoparticles migrate towards the magnetic force.

Figure 6 illustrates the core/shell approach to magnetic gold nanoparticles.

Figure 7 illustrates a comparison of the relative stabilities of Ag, Ag/Au alloy, and Ag@Au core/shell nanoparticle-DNA conjugates at different salt concentrations.

## **DETAILED DESCRIPTION OF THE INVENTION:**

In one aspect the present invention provides for core/shell nanoparticles, comprising a nanoparticle core and a gold shell. The core material can comprise any nanoparticle known to those of skill in the art including, but not limited to, metal, semiconductor, and magnetic nanoparticles. In a preferred embodiment, the core material is comprised of metal or magnetic nanoparticles including, but not limited to, Ag, Pt, Fe, Co, Ni, FePt, FeAu,  $\text{Fe}_3\text{O}_4$ , and  $\text{Co}_3\text{O}_4$ . Methods for preparing such nanoparticles are well known in the art. For example, see, *e.g.* Schmid, G. (ed.) *Clusters and Colloids* (VCH, Weinheim, 1994); Hayat, M.A. (ed.) *Colloidal Gold: Principles, Methods, and Applications* (Academic Press, San Diego, 1991); Massart, R., *IEEE Transactions On Magnetics*, **17**, 1247 (1981); Ahmadi, T.S. et al., *Science*, **272**, 1924 (1996); Henglein, A. et al., *J. Phys. Chem.*, **99**, 14129 (1995); Curtis, A.C., et al., *Angew. Chem. Int. Ed. Engl.*, **27**, 1530 (1988).

In yet another aspect the present invention provides a method for preparation of non-alloying gold core/shell nanoparticles and product produced therefrom. The method of the

invention comprises providing an inner nanoparticle core, treating the core simultaneously with a solution comprising a gold salt and a solution comprising a reducing agent, and isolating the core/shell nanoparticles. The method provides for the first time a non-alloying gold shell surrounding a nanoparticle core. These non-alloying gold core/shell nanoparticles exhibit  
5 surprising superior spectroscopic properties not found in conventional gold core/shell nanoparticles and can be functionalized with molecules such as nucleic acids and receptors, to produce nanoparticle conjugates that can be used for targeting and detecting target analytes such as nucleic acids, antigens, proteins, carbohydrates and other substances.

In practicing the method of the invention, the method can be performed at any  
10 temperature favorable in producing a non-alloying gold shell surrounding the core. Generally, the temperature depends on the choice of reaction solvent used to generate the gold shell. Suitable, but non-limiting, examples of reaction solvents include water, aqueous buffer solutions, oleic acid and trioctylphosphine oxide. In practicing this invention, trisodium citrate solution is preferred.

In practicing the method of the invention, the temperature generally ranges from about 0  
15 °C to about 45 °C in water or aqueous reaction solutions. For organic solvents, the temperature generally ranges from about 130°C to about 180°C when oleic acid and trioctylphosphine oxide are used.

The gold salt can comprise any suitable gold salt including, but not limited to,  $\text{HAuCl}_4$ ,  
20  $\text{NaAuCl}_4$ ,  $\text{KAuCl}_4$ , or  $\text{KAu}(\text{CN})_2$ . In practicing the invention, the preferred gold salt is  $\text{HAuCl}_4$ .

The reducing agent can comprise any suitable reducing agent capable of reducing the valency of the gold that comprises the gold salt solution including, but not limited to,  $\text{NaBH}_4$ , ascorbic acid,  $\text{NH}_2\text{OH}$  and  $\text{N}_2\text{H}_4$ . In practicing the invention, the preferred reducing agent is  
25  $\text{NaBH}_4$ .

In one aspect of the invention, the core/shell nanoparticles have specific binding substances bound to the gold shell surrounding the nanoparticle. The specific binding substance may be natural and synthetic nucleic acids, natural and synthetic polypeptides, antibodies, Fab and Fab' antibody fragments, biotin, avidin and haptens such as digoxin. Those  
30 skilled in these arts will recognize a wide variety of specific binding substances that can be linked to the gold shell surrounding the nanoparticles.

In another aspect, the present invention provides for core/shell nanoparticle oligonucleotide conjugates, comprising a nanoparticle core, a gold shell surrounding the

nanoparticle, and an oligonucleotide attached to the gold surface of the core/shell nanoparticle. Any suitable method for attaching oligonucleotides onto a gold surface may be used. A particularly preferred method for attaching oligonucleotides onto a gold surface is based on an aging process described in U.S. application nos. 09/344,667, filed June 25, 1999; 09/603,830, filed June 26, 2000; 09/760,500, filed January 12, 2001; 09/820,279, filed March 28, 2001; 09/927,777, filed August 10, 2001; and in International application nos. PCT/US97/12783, filed July 21, 1997; PCT/US00/17507, filed June 26, 2000; PCT/US01/01190, filed January 12, 2001; PCT/US01/10071, filed March 28, 2001, the disclosures which are incorporated by reference in their entirety. The aging process provides nanoparticle-oligonucleotide conjugates with unexpected enhanced stability and selectivity. The method comprises providing oligonucleotides preferably having covalently bound thereto a moiety comprising a functional group which can bind to the nanoparticles. The moieties and functional groups are those that allow for binding (*i.e.*, by chemisorption or covalent bonding) of the oligonucleotides to nanoparticles. For instance, oligonucleotides having an alkanethiol, an alkanedisulfide or a cyclic disulfide covalently bound to their 5' or 3' ends can be used to bind the oligonucleotides to a variety of nanoparticles, including gold nanoparticles.

The oligonucleotides are contacted with the nanoparticles in water for a time sufficient to allow at least some of the oligonucleotides to bind to the nanoparticles by means of the functional groups. Such times can be determined empirically. For instance, it has been found that a time of about 12-24 hours gives good results. Other suitable conditions for binding of the oligonucleotides can also be determined empirically. For instance, a concentration of about 10-20 nM nanoparticles and incubation at room temperature gives good results.

Next, at least one salt is added to the water to form a salt solution. The salt can be any suitable water-soluble salt. For instance, the salt may be sodium chloride, magnesium chloride, potassium chloride, ammonium chloride, sodium acetate, ammonium acetate, a combination of two or more of these salts, or one of these salts in phosphate buffer. Preferably, the salt is added as a concentrated solution, but it could be added as a solid. The salt can be added to the water all at one time or the salt is added gradually over time. By "gradually over time" is meant that the salt is added in at least two portions at intervals spaced apart by a period of time. Suitable time intervals can be determined empirically.

The ionic strength of the salt solution must be sufficient to overcome at least partially the electrostatic repulsion of the oligonucleotides from each other and, either the electrostatic attraction of the negatively-charged oligonucleotides for positively-charged nanoparticles, or

the electrostatic repulsion of the negatively-charged oligonucleotides from negatively-charged nanoparticles. Gradually reducing the electrostatic attraction and repulsion by adding the salt gradually over time has been found to give the highest surface density of oligonucleotides on the nanoparticles. Suitable ionic strengths can be determined empirically for each salt or combination of salts. A final concentration of sodium chloride of from about 0.1 M to about 1.0 M in phosphate buffer, preferably with the concentration of sodium chloride being increased gradually over time, has been found to give good results.

After adding the salt, the oligonucleotides and nanoparticles are incubated in the salt solution for an additional period of time sufficient to allow sufficient additional oligonucleotides to bind to the nanoparticles to produce the stable nanoparticle-oligonucleotide conjugates. As will be described in detail below, an increased surface density of the oligonucleotides on the nanoparticles has been found to stabilize the conjugates. The time of this incubation can be determined empirically. A total incubation time of about 24-48, preferably 40 hours, has been found to give good results (this is the total time of incubation; as noted above, the salt concentration can be increased gradually over this total time). This second period of incubation in the salt solution is referred to herein as the "aging" step. Other suitable conditions for this "aging" step can also be determined empirically. For instance, incubation at room temperature and pH 7.0 gives good results.

The conjugates produced by use of the "aging" step have been found to be considerably more stable than those produced without the "aging" step. As noted above, this increased stability is due to the increased density of the oligonucleotides on the surfaces of the nanoparticles which is achieved by the "aging" step. The surface density achieved by the "aging" step will depend on the size and type of nanoparticles and on the length, sequence and concentration of the oligonucleotides. A surface density adequate to make the nanoparticles stable and the conditions necessary to obtain it for a desired combination of nanoparticles and oligonucleotides can be determined empirically. Generally, a surface density of at least 10 picomoles/cm<sup>2</sup> will be adequate to provide stable nanoparticle-oligonucleotide conjugates. Preferably, the surface density is at least 15 picomoles/cm<sup>2</sup>. Since the ability of the oligonucleotides of the conjugates to hybridize with nucleic acid and oligonucleotide targets can be diminished if the surface density is too great, the surface density is preferably no greater than about 35-40 picomoles/cm<sup>2</sup>.

As used herein, "stable" means that, for a period of at least six months after the conjugates are made, the nanoparticles remain dispersed, a majority of the oligonucleotides

remain attached to the nanoparticles, and the oligonucleotides are able to hybridize with nucleic acid and oligonucleotide targets under standard conditions encountered in methods of detecting nucleic acid and methods of nanofabrication.

In yet a further aspect the invention provides methods for the detection of a target analytes such as nucleic acids comprising contacting the core/shell nanoparticle oligonucleotide conjugates of the instant invention with a target nucleic acid sequence under conditions that allow hybridization between at least a portion of the oligonucleotides bound to the nanoparticle and at least a portion of the target nucleic acid sequence. In addition, protein receptors and other specific binding pair members can be functionalized with oligonucleotides and immobilized onto oligonucleotide-modified nanoparticles to generate a new class of hybrid particles (nanoparticle-receptor conjugates) that exhibit the high stability of the oligonucleotide modified particles but with molecular recognition properties that are dictated by the protein receptor rather than DNA. Alternatively, one could functionalize a protein that has multiple receptor binding sites with receptor-modified oligonucleotides so that the protein receptor complex could be used as one of the building blocks, in place of one of the inorganic nanoparticles, in the original nanomaterials assembly scheme discussed above. The use of these novel nanoparticle-receptor conjugates in analyte detection strategies have been evaluated in a number of ways including identification of targets and screening for protein-protein interactions. For suitable hybridization conditions for nucleic acid detection, and methods for preparing nanoparticle-receptor conjugates are described in U.S. application nos. 09/344,667, filed June 25, 1999; 09/603,830, filed June 26, 2000; 09/760,500, filed January 12, 2001; 09/820,279, filed March 28, 2001; 09/927,777, filed August 10, 2001; and in International application nos. PCT/US97/12783, filed July 21, 1997; PCT/US00/17507, filed June 26, 2000; PCT/US01/01190, filed January 12, 2001; PCT/US01/10071, filed March 28, 2001, the disclosures which are incorporated by reference in their entirety. Once a core/shell nanoparticle conjugate of the invention binds to a target molecule, a change in the optical characteristics of the core/shell nanoparticle conjugates can be readily detected. In another embodiment the detection step is performed in the presence of an applied magnetic field which further enhances hybridization or binding of the nanoparticle conjugate with the target molecule such as a nucleic acid .

The invention further provides a method of nanofabrication based on the core-shell nanoparticle conjugates of the invention. Nanostructures and methods for prepare the materials from nanoparticles have been described in U.S. application nos. 09/344,667, filed June 25,



1999; 09/603,830, filed June 26, 2000; 09/760,500, filed January 12, 2001; 09/820,279, filed March 28, 2001; 09/927,777, filed August 10, 2001; and in International application nos. PCT/US97/12783, filed July 21, 1997; PCT/US00/17507, filed June 26, 2000; PCT/US01/01190, filed January 12, 2001; PCT/US01/10071, filed March 28, 2001, the disclosures which are incorporated by reference in their entirety. The method comprises providing at least one type of linking oligonucleotide having a selected sequence, the sequence of each type of linking oligonucleotide having at least two portions. The method further comprises providing one or more types of core/shell nanoparticles having oligonucleotides attached thereto, the oligonucleotides on each type of nanoparticles having a sequence complementary to a portion of the sequence of a linking oligonucleotide. The linking oligonucleotides and nanoparticles are contacted under conditions effective to allow hybridization of the oligonucleotides on the nanoparticles to the linking oligonucleotides so that a desired nanomaterials or nanostructure is formed.

The invention provides another method of nanofabrication. This method comprises providing at least two types of core-shell nanoparticles of the invention having oligonucleotides attached thereto. The oligonucleotides on the first type of nanoparticles have a sequence complementary to that of the oligonucleotides on the second type of nanoparticles. The oligonucleotides on the second type of nanoparticles have a sequence complementary to that of the oligonucleotides on the first type of nanoparticle-oligonucleotide conjugates. The first and second types of nanoparticles are contacted under conditions effective to allow hybridization of the oligonucleotides on the nanoparticles to each other so that a desired nanomaterials or nanostructure is formed.

The invention further provides nanomaterials or nanostructures composed of core-shell nanoparticles having oligonucleotides attached thereto, the nanoparticles being held together by oligonucleotide connectors.

The following examples serve to illustrate certain embodiments of the present invention, and do not limit it in scope or spirit. Certain obvious alternatives and variations will be apparent to those of skill in the art.

#### Example 1: Synthesis of Ag/Au core/shell nanoparticles prepared via a two-step synthesis

This Example illustrates the inventive process for preparing Ag/Au core/shell nanoparticles. In part A, methods for preparing silvercores are described. In part B, a method for preparing Ag/gold core/shell nanoparticles is provided. Silver nanoparticles are desired

compositions for building blocks in material synthesis and as biological labels for two important reasons: (1) silver particles exhibit a surface plasmon band between ~390 and 420 nm, depending on the particle size;<sup>11</sup> this is a spectral regime that is distinct from that of gold(520-580 nm). (2) The extinction coefficient of the surface plasmon band for a silver particle is approximately 4 times as large as that for an gold particle of the same size.<sup>12</sup> Therefore, silver particles functionalized with DNA would provide not only an opportunity to tailor the optical properties of DNA/nanoparticle composite structures but also routes to new diagnostic systems that rely on the position and intensity of the surface plasmon band (e.g. colorimetric systems based on absorption or scattering, or SPR and SERS detection systems).

Experimentally, it has been determined that silver nanoparticles cannot be effectively passivated by alkylthiol-modified-oligonucleotides using the established protocols for modifying goldparticles.<sup>2</sup> Indeed, silver particles prepared via such methods irreversibly aggregate when heated in a solution with a salt concentration necessary to effect DNA hybridization (0.05 M NaCl or greater). Herein, a core/shell approach was applied to overcome this problem. In this approach, a thin goldshell was grown upon a silver nanoparticle, forming a particle with a gold outer surface that can be easily modified with alkylthiol-oligonucleotides. This approach could be generalized to prepare other particles such as Cu and Pt to create a series of core/shell particles with tailorable physical properties by virtue of choice of core but the surface chemistry and stability of the native, and oligonucleotide modified, pure gold particles.

#### A. Preparation of silver nanoparticle cores

Silver nanoparticles were synthesized silver nanocrystals by reduction of silver nitrate by sodium borohydride in a trisodium citrate solution. Two methods for synthesizing the silver nanocrystals are described below and the resulting core nanocrystals are compared.

Method No. 1: AgNO<sub>3</sub> (2.2 mg) and sodium citrate dihydrate (8.2 mg) were dissolved in 99 ml of Nanopure water in a 250-ml flask. With stirring and under Ar, this flask was placed in a ice bath for 15 min. Then 1 ml of sodium borohydride solution (0.14 M) was injected into the solution. After stirring for 1 hr, the solution was warmed to room temperature. The silver nanoparticles (~ 12 nm in diameter) were obtained. Without further purification, these silver nanoparticles could be directly used for the gold shell growth.

Method No. 2: AgNO<sub>3</sub> (2.2 mg) and sodium citrate dihydrate (8.2 mg) were dissolved in 98 ml of Nanopure water in a 250-ml flask. With stirring and under an Ar atmosphere, this flask was placed in an ice bath for 15 min. Then 1 ml of sodium borohydride solution (0.14

M) was injected into the solution. After stirring for 1 hr, the solution was warmed to room temperature. The Ag nanoparticles (~ 12 nm in diameter) were obtained. Bis(p-sulfonatophenyl)-phenylphosphine (BSPP, 17 mg) was put into the silver nanoparticle solution and stirred overnight. The silver nanoparticles were subsequently purified and isolated by gradient centrifugation between 12 kRPM ~ 20 kRPM. The resulting silver nanoparticle-containing aliquots from the precipitation were combined, and dispersed in Nanopure water.

Comparison results: Silver particles prepared by method no. 2 have better size distribution compared with those prepared by method no. 1 ( $\sigma=18\%$  for method no. 2;  $\sigma=30\%$  for method no. 1). Subsequent studies have shown, however, that silver particles prepared by either method serve well as cores for generating silver/gold core/shell nanoparticles.

#### B. Preparation of silver/gold core/shell nanoparticles

This step describes gold shell growth on the surface of silver cores described above. For silver nanoparticles, gold shells were grown on the silver core surface by reduction of HAuCl<sub>4</sub> with the reducing silverent NaBH<sub>4</sub>. The reduced gold has affinity for the silver surface, in part, because of the low surface chemical potential of the silver nanoparticles and near-zero lattice mismatch between these two materials. Two methods for growing gold shells on silver core nanocrystals are described below and the resulting core/shell nanoparticles were compared. silver core particles were prepared by method no. 1 described above.

Method No. 1: Gold shells (approximately one-monolayer thick) were grown on the surface of the silver nanoparticles (0.25 nmol of silver particles in 100 ml of 0.3 mM sodium citrate aqueous solution) by simultaneous dropwise addition, at a rate of between about 50  $\mu$ L ~ 600  $\mu$ L/min., of HAuCl<sub>4</sub> and NaBH<sub>4</sub> solutions (in Nanopure water) at 0 °C to the silver nanoparticle suspension. The simultaneous dropwise addition of dilute gold precursors inhibits the formation of gold cluster nucleation sites by keeping the concentration of these gold forming reagents at about 2  $\mu$ M. After enough HAuCl<sub>4</sub> and NaBH<sub>4</sub> was added to the nanoparticles to produce one monolayer of gold on the particles (see Equation 1 for a calculation of shell thickness), addition was halted.

Equation 1:

$$V_{\text{core}} = \frac{4}{3} \pi R^3;$$

$$V_{\text{core/shell}} = \frac{4}{3} \pi (R+a)^3,$$

wherein  $a$  is the shell thickness, (0.3 nm for 1 monolayer of Au);

$$V_{\text{shell}} = V_{\text{core/shell}} - V_{\text{core}};$$

$$N_{\text{shell}} = d_{\text{shell}} \cdot V_{\text{shell}} / \text{FW}_{\text{shell}};$$

wherein,  $V_{\text{shell}}$  is volume of shell;

$N_{\text{shell}}$  is the amount in mole of the shell;

$d_{\text{shell}}$  is density of shell materials, (for gold,  $d=19.3$  g/ml);

$\text{FW}_{\text{shell}}$ , the formula weight of shell materials, (for gold,  $\text{FW}=196.97$  amu)

Gold was added 5 % excess, calculated assuming 12-nm spheres: 0.8 mg of  $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$  and 3.7 mg of  $\text{NaBH}_4$ . Once 5% excess was achieved, addition of the solutions was stopped (halting formation of the shell) and 30  $\mu\text{mol}$  of Bis(*p*-sulfonatophenyl)phenylphosphine (BSPP) was added. The silver/gold core/shell nanoparticles were then purified by centrifugation and dispersed in Nanopure water (12.4 nm in diameter, ( $\sigma=18\%$ )), giving a 96% yield and a ratio of silver to gold of about 5.5:1.

Method No. 2: Gold shells (approximately one-monolayer thick) were grown on the surface of the silver nanoparticles (0.25 nmol of silver particles in 100 ml of 0.3 mM sodium citrate aqueous solution) by simultaneously treating them with  $\text{HAuCl}_4$  (2 mM) and  $\text{NaBH}_4$  (6 mM) via dropwise addition at room temperature at a rate of between about 50  $\mu\text{L}$  ~ 600  $\mu\text{L}/\text{min}$ . The simultaneous dropwise addition of dilute gold precursors inhibits the formation of gold cluster nucleation sites by keeping the concentration of these gold forming reagents at about 2  $\mu\text{M}$ . After sufficient  $\text{HAuCl}_4$  and  $\text{NaBH}_4$  were added to the nanoparticles to produce one monolayer of gold on the particles (5 % excess, calculated assuming 12-nm spheres: 0.8 mg of  $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$  and 3.7 mg of  $\text{NaBH}_4$ ), the reaction was stopped and 30  $\mu\text{mol}$  of BSPP was added. The silver/gold core/shell nanoparticles were then purified by centrifugation and dispersed in nanopure water, giving a weight percent yield of about 90%, and average particle size of about 12.5 nm, and an silver to gold ratio of about 6.3:1.

Comparison results: The core/shell nanoparticles produced via method no. 1 (synthesis at 0 °C) were found to have better stability in 0.5 M NaCl solution compared to core/shell nanoparticles produced by method no. 2 (synthesis at room temperature). This

result may be due, in part, to a slower rate of shell growth at 0 °C than the growth rate at room temperature.

(c) Discussion

5 Silver nanoparticles were prepared by literature methods.<sup>13</sup> The particles were then passivated with BSPP (0.3 mM), purified by gradient centrifugation (collecting the primary fraction; ~12 nm in diameter), and dispersed in Nanopure water. Gold shells, approximately one-monolayer thick, were grown on the surface of the silver nanoparticles (0.32 nmol of silver particles in 100 mL of 0.3 mM sodium citrate aqueous solution) by simultaneously  
10 treating them with HAuCl<sub>4</sub> and sodium borohydride via dropwise addition at 0 °C. The reduced gold has an affinity for the silver surface, in part, because of its near zero lattice mismatch.<sup>14</sup> The simultaneous dropwise addition of dilute gold precursors inhibits the formation of gold cluster nucleation sites by keeping the concentration of these gold forming reagents at about 2 μM. After enough HAuCl<sub>4</sub> and NaBH<sub>4</sub> were added to the nanoparticles to  
15 produce one monolayer of gold on the particles (5% excess, calculated assuming 12-nm spheres: 0.8 mg of HAuCl<sub>4</sub> · 3H<sub>2</sub>O and 3.7 mg of NaBH<sub>4</sub>), the reaction was stopped and 30 mM of BSPP was added. Then, the silver/gold core/shell nanoparticles were purified by centrifugation and dispersed in nanopure water (12.4 nm in diameter particles, (σ = 18%). Figure 1A shows a TEM image of silver/gold core/shell nanoparticles which was obtained  
20 using a Hitachi 8100 electron microscopy. A typical TEM sample was prepared by depositing one drop of nanoparticles solution onto a carbon coated copper grid. The excess solution was wicked away by filter paper and dry in vacuum. The silver:gold ratio in these core/shell particles was determined to be 5.2:1 by energy dispersive X-ray (EDX) microanalysis of the particles (Figure 1B). Figure 1B illustrates an EDX spectrum of silver  
25 core particles (dotted line) and silver/gold core/shell particles (solid line). L and M signify electron transitions into the L and M shell of the atoms, respectively, from higher states. EDX analysis was performed on a field emission scanning-electron microscopy (FESEM) Hitachi 4500. The SEM samples were prepared by depositing of one drop of nanoparticle solution on a silicon plate. The silver:gold ratio corresponds to an gold shell thickness of 3.1  
30 +/- 0.6 Å, which correlates with approximately one monolayer of gold atoms.

Significantly, the extinction spectrum of the core/shell particles is very similar to that for the citrate-stabilized pure silver particles. The surface plasmon band of the silver remains

at the same wavelength but is dampened by about 10%, and the gold plasmon band is observed as a slight buckle at 500 nm. These spectral features provide strong evidence for gold shell growth. It should be noted that using different procedures, others have prepared gold-coated silver nanoparticles.<sup>15</sup> However, those procedures lead to silver/gold alloys;<sup>15a</sup> the extinction spectra of such particles exhibit characteristic red shifting and *broadening* of the plasmon resonance. Moreover, if one intentionally makes a solution of alloyed silver/gold particles, they can be easily distinguished from core/shell particles with comparable silver/gold ratios (see Supporting Information). Indeed, the core/shell silver/gold nanoparticles prepared by the methods of the instant invention retain the optical properties of the core with no observed red shifting of the silver plasmon band, (Figure 1C). Using Mie theory, the extinction spectrum of a particle consisting of an 11.8 nm silver core and a monolayer gold shell was calculated.<sup>11</sup> The calculated spectrum was almost superimposable with the experimentally measured spectrum of the particles, (Figure 1C, inset). Figure 1C illustrates the UV-visible spectra of silver core (dotted line) and silver/gold core/shell (solid line) wherein the inset shows the calculated extinction spectra of silver particles (dotted line) and silver/gold core/shell particles (solid line). The UV/Vis spectra were obtained using a HP 8453 diode array spectrophotometer.

#### Example 2: Preparation of silver/gold core/shell Nanoparticle-oligonucleotide Conjugates

This Example describes the preparation of silver/gold core/shell nanoparticle oligonucleotide conjugates as probes for detecting a target nucleic acid. Two methods were employed and the resulting probes were then compared for stability. The oligonucleotide sequences used in making the conjugates are shown in Figure 2a. These sequences were synthesized using standard phosphoramidite chemistry according to the literature. (James J. Storhoff, Robert Elghanian, Robert C. Mucic, Chad A. Mirkin, and Robert L. Letsinger, *J. Am. Chem. Soc.*, **1998**, 120, 1959).

##### (a) Preparation of core/shell nanoparticle conjugates

Method No. 1: Nanoparticle probes with appropriate probe oligonucleotides were prepared by derivatizing 10 mL of aqueous core/shell nanoparticle colloid (from method no. 1) with 8~10 OD (in about 500  $\mu$ L) of alkanethiol-oligonucleotide (final oligonucleotide concentration is about 2  $\mu$ M). After standing overnight (about 15 h), the solution was brought

to 10 mM phosphate buffer (pH 7), using 100 mM concentrated phosphate stock buffer, and salt (from a 2 M aqueous NaCl solution) added to 0.05 M NaCl after 0.5 h, allowed to stand for about 8 h, then further addition of NaCl to 0.1 M, and after another standing time of about 8 h, another addition of NaCl to about 0.3 M and allowed to stand for a final ~8 h. To remove excess DNA, colloids were centrifuged for 30 min at 18,000 rpm using 1.5 mL eppendorf tubes. Following removal of the supernatant, the oily precipitate was washed with a volume equal to the discarded supernatant with 0.3 M NaCl, 10 mM phosphate buffer (pH 7) solution, centrifuged, and dispersed in 0.3 M NaCl, 10 mM phosphate buffer (pH 7), 0.01% azide solution. The final colloids were refrigerated and stored for later use.

Method No. 2: Nanoparticle probes with appropriate probe oligonucleotides were prepared by derivatizing 10 mL of aqueous colloid with 8~10 OD of alkanethiol-oligonucleotide (final oligonucleotide concentration is about 2  $\mu$ M). After standing overnight (~15 h), the solution was brought to 10 mM phosphate buffer (pH 7), using 100 mM concentrated phosphate stock buffer, and salt added to 0.1 M NaCl, allowed to stand for about 20 h, and again, salt added to 0.3 M after another ~8 h. The mixture was allowed to stand for about 4 to 8 hours. To remove excess DNA, colloids were centrifuged for 30 min at 18,000 rpm using 1.5 mL eppendorf tubes. Following removal of the supernatant, the oily precipitate is washed with 0.3 M NaCl, 10 mM phosphate buffer (pH 7) solution in the same volume as the discarded supernatant, centrifuged, and dispersed in 0.3 M NaCl, 10 mM phosphate buffer (pH 7), 0.01% azide solution. The final colloids were refrigerated and stored for later use.

#### (b) Evaluation of stability of core/shell nanoparticle oligonucleotide conjugates

The core/shell nanoparticle oligonucleotide conjugates prepared by the two methods described above were compared using a salting procedure as described in each of the above 2 methods.

By method 1, the salt concentration was increased from 0.05 M NaCl to 0.1 M NaCl, and then to 0.3 M NaCl. By method 2, the salt concentration was increased in two steps: directly to 0.1 M NaCl and then to 0.3 M NaCl. Method 1 generates a higher quality nanoparticle-oligonucleotide conjugate when compared with those prepared by method 2. Via method 2, about 15% of the nanoparticle-oligonucleotide conjugates are not of adequate quality. Core/shell nanoparticle-oligonucleotide conjugate quality is evaluated by UV-Vis

spectroscopy. Acceptable quality conjugates show a UV-Vis spectrum with the surface plasmon absorption peak centering at 400 nm, while poor (inadequate) quality conjugates show an absorption peak which is significantly damped and red-shifts to 450-550 nm.

(c) Discussion

The surface modification of these core/shell nanoparticles with 3'- and 5'-alkanethiol-capped oligonucleotides was accomplished using a procedure identical to the one used for 13-nm gold particles.<sup>2d</sup> Significantly, the oligonucleotide-modified core/shell particles exhibit the stability of oligonucleotide modified particles prepared using pure gold nanoparticles and can be suspended in 1M NaCl solution indefinitely. This represents a significant advantage over the oligonucleotide modified silver/gold alloy particles which irreversibly aggregate under comparable solution conditions and do not exhibit the stability of the oligonucleotide-modified core/shell particles of the instant invention.

Moreover, the core/shell particles undergo hybridization with complementary linking oligonucleotides to form aggregated structures with a concomitant darkening of the solution; (Figure 2). Like the oligonucleotide modified pure gold nanoparticles, the nanoparticles comprising these silver/gold core/shell aggregate structures can be disassembled by heating the aggregates above the "melting temperature" ( $T_m$ ) of the duplex linkers (Figure 1 D). UV-vis spectroscopy shows a red-shifting and dampening of the plasmon resonance of the core/shell particles upon DNA-induced assembly, (Figure 1D, inset). Figure 1D illustrates the thermal denaturation ("melting") curve of aggregates formed from hybridized oligonucleotide modified silver/gold core/shell particles in buffer solution (0.3 M NaCl and 10 mM phosphate buffer, pH=7). The oligonucleotide sequences are provided in Figure 2A. The Figure 1D inset shows the UV-visible spectra of dispersed oligonucleotide-modified silver/gold core/shell particles (solid line) and aggregated (dotted line) oligonucleotide-modified silver/gold core/shell particles formed via hybridization. UV-Vis spectra of silver and silver/gold core/shell particles (Figure 1C and inset of Figure 1D) were obtained using a HP 8453 diode array spectrophotometer. The thermal denaturation experiment (Figure 1D) was performed using an HP 8453 diode array spectrophotometer equipped with a HP 89090a Peltier temperature controller. The UV-Vis signature of the silver/gold core/shell probe/target oligonucleotide aggregates was recorded at 1 min intervals, as the temperature was increased from 25 to 70 °C with a holding time of 1 min/deg.



The particle assembly process induced by the complementary DNA also can be monitored on a C<sub>18</sub>-reverse-phase alumina TLC plate, allowing for comparison with the pure gold system. The spot test results shown in Figure 2b and 2c were obtained as follows: a solution of the appropriate oligonucleotide target (24 pmol, 3  $\mu$ L) was added to a 600  $\mu$ L thin-wall PCR tube containing 200  $\mu$ L of each silver/gold core/shell nanoparticle-oligonucleotide conjugates. After standing for 30 min at room temperature, the solution was transferred to a temperature controlled electro thermal heater. After the set-point temperature was reached (monitored with an ethanol thermometer, 0.5  $^{\circ}$ C increments), the mixture was allowed to equilibrate for 5 min at which time 2.5  $\mu$ L aliquots of the silver-gold probe/target oligonucleotide solution were transferred with a pipet onto the reverse-phase alumina plate and allowed to dry.

As shown in Figure 2, with the core shell particles, a distinct yellow-to-dark brown color change is observed upon particle assembly in the presence of complementary target, Figure 2B-I and 2B-II. Note that when the solution temperature is above the  $T_m$  of the DNA duplex linkers, a yellow spot is formed on the reverse phase alumina support, Figure 2B-III. When one compares the properties of these new silver/gold core/shell probes with those derived from pure gold nanoparticles (with identical oligonucleotide sequences), Figure 2C, one realizes that the core/shell particles provide a route to a second colorimetric change distinct from the gold system that ultimately could be used for monitoring two different oligonucleotide targets in one sample. Such capabilities could be important for both research-based and clinical genomic assays where multicolor formats are essential.<sup>16</sup>

Example 3: Comparison of silver, silver/gold core/shell and silver/gold alloy nanoparticle oligonucleotide conjugates

In this Example, the silver/gold core/shell nanoparticles prepared as described in Example 1 (method no. 1) were compared to gold nanoparticles<sup>2</sup> and to silver/gold alloy nanoparticles.

The silver/gold alloy nanoparticles were prepared by the method of Wang, Z. L.; El-Sayed, M. A. *J. Phys. Chem.B* **1999**, *103*, 3529. Following literature procedure, 0.8 mg of HAuCl<sub>4</sub>•3H<sub>2</sub>O and 1.8 mg of silverNO<sub>3</sub> were dissolved in 95 ml of nanopure water. The solution was heated to reflux, and 5 ml of 1% sodium citrate was added to the solution. After refluxing an additional 30 min., the solution was allowed to cool to room temperature.

The UV-Vis spectrum of the alloy particles exhibits a surface plasmon band at 428 nm with a full width at half-maximum (FWHM) of 90 nm (0.62 eV). In contrast, the UV-Vis spectrum of the silver/gold core/shell nanoparticle, with a comparable silver/gold ratio, exhibits a surface plasmon band at 400 nm with a FWHM of 58 nm (0.45 eV). Figure 7 shows a comparison of the relative stabilities of Ag, Ag/Au alloy, and Ag/Au core/shell nanoparticle-DNA conjugates at different salt concentrations. The surface plasmon bands were monitored at 400 nm for Ag and Ag/Au particles, and at 430 nm for Ag/Au alloy particles, respectively.

The surface modification of these core/shell and alloy nanoparticles with 3'- and 5'-alkanethiol-capped oligonucleotides was accomplished using a procedure identical to the one used for 13-nm gold particles. See Storhoff, J. J.; Elghanian, R.; Mucic, R. C.; Mirkin, C. A.; Letsinger, R. L. *J. Am. Chem. Soc.* **1998** *120*, 1959. Significantly, the oligonucleotide-modified core/shell nanoparticles exhibit the stability of the pure gold nanoparticles and can be suspended in 1M NaCl solutions indefinitely. In contrast, the oligonucleotide-modified silver/gold alloy particles irreversibly aggregate when brought to a salt concentration of 0.1 M.

Another way to evaluate stability of the particle/DNA conjugate uses a DNA melting test. The core/shell nanoparticle/DNA conjugate can reversibly hybridize with target DNA in a salt concentration range from 0.1 to 1.0 M, and the resulting nanoparticle aggregates can "melt" off when heated above the melting temperature. This hybridization/dehybridization process is completely reversible for core/shell particles. The core/shell particle/DNA conjugates show no degradation after 100 cycles. In sharp contrast, the silver/gold-alloy particle/DNA conjugates irreversibly aggregate even under minimal salt concentrations (~0.05 M NaCl) necessary to effect hybridization of oligonucleotides.

#### Example 4: Preparation of Pt/gold core/shell nanoparticles

This Example describes the preparation of Pt/gold core/shell nanoparticles by the inventive process. In Part A, Pt core nanoparticles were prepared by hydrogen reduction of  $K_2PtCl_4$  in an overnight reaction. In Part B, goldshells were grown on the Pt cores.

##### (a) Preparation of Pt core nanoparticles

In a 500-ml three-neck flask,  $K_2PtCl_4$  (8.3 mg) and sodium polyacrylate (20 mg) were dissolved in 200 ml of Nanopure water.  $H_2$  was bubbled into the reaction solution overnight with stirring. This resulted in Pt nanoparticles that were purified and isolated, yielding nanoparticles of about 12 nm in diameter.

(b) Preparation of Pt/gold core/shell nanoparticles

100 ml of 12-nm Pt nanoparticle solution (as prepared according to the above procedure) was put into a 250-ml three-neck flask. To the nanoparticle solution were added  $HAuCl_4$  and  $NaBH_4$  dropwise, simultaneously, at 0 °C. The simultaneous dropwise addition of dilute goldprecursors inhibits the formation of gold cluster nucleation sites by keeping the concentration of these gold forming reagents at about 2  $\mu$ M. After sufficient amounts of  $HAuCl_4$  and  $NaBH_4$  were added to the nanoparticles to produce one monolayer of gold on the Pt nanoparticles (5 % excess, calculated assuming 12-nm spheres: 16 mg of  $HAuCl_4 \cdot 3H_2O$  and 8 mg of  $NaBH_4$ ), addition of these reagents to the reaction was stopped. UV-Vis spectra of Pt core and Pt/gold core/shell nanoparticle are shown in **Figure 3**.

Example 5: Preparation of magnetic  $Fe_3O_4$ /gold core/shell nanoparticles

This Example describes the preparation of magnetic gold nanoparticles by the inventive process. In Part A,  $Fe_3O_4$  magnetic core nanoparticles were prepared. In Part B, goldshells were grown on the magnetic cores. Other magnetic cores could be used in place of  $Fe_3O_4$  such as Co, Fe, Ni, FePt, and FeAu. Figure 6 illustrates the core/shell approach to preparing magnetic gold nanoparticles.

(a) Preparation of  $Fe_3O_4$  core nanoparticles

In a typical synthesis,  $Fe_3O_4$  nanoparticles were prepared as follows. First, 0.86 g  $FeCl_2 \cdot 4H_2O$  and 2.35 g  $FeCl_3 \cdot 6H_2O$  were dissolved in 50 mL nanopure water under an inert  $Ar_{(g)}$  atmosphere. The solution was heated to 80 °C with vigorous stirring. A solution of 100 mg of neat decanoic acid in 5 mL of acetone was added to the Fe solution, followed by 5 mL of 28% (w/w)  $NH_3/H_2O$ . Additional neat decanoic acid was added to the suspension in 5x 0.2 g amounts over 5 min. The reaction was allowed to proceed for 30 min at 80 °C with stirring to produce a stable, water-based suspension. Following formation of the suspension, the reaction was cooled slowly to room temperature. The resulting  $Fe_3O_4$  nanoparticles (5.0 nmol) were treated further with  $Na_2S$  (8.0 mg) solution overnight to allow for sulfur exchange

at the particle surface. Sulfur ions replace oxygen on the surface of the  $\text{Fe}_3\text{O}_4$  nanoparticles, providing the growth site for the goldshell. This sulfur exchange process is also necessary for the preparation of  $\text{Co}_3\text{O}_4$  magnetic cores.

(b) Preparation of  $\text{Fe}_3\text{O}_4$ /gold core/shell nanoparticles

The procedure for growing goldshell is similar to that of core/shell silver/goldpreparation described in Example 1. The UV-Vis spectrum, of the  $\text{Fe}_3\text{O}_4$ /gold shell growth is shown in **Figure 4**. **Figure 5** illustrates that in an applied magnetic field,  $\text{Fe}_3\text{O}_4$ /gold core/shell particles behave as super paramagnetic particles. **Figure 5** illustrates that the gold nanoparticles become colorless after 12 hours in a magnetic field.

Example 6: Preparation of magnetic Co/gold core/shell nanoparticles

This Example describes the preparation of magnetic gold nanoparticles by the inventive process. In Part A, Co magnetic core nanoparticles were prepared. In Part B, goldshells were grown on the Co magnetic cores.

(a) Preparation of Co nanoparticle cores

O-dichlorobenzene (15.9 g), trioctylphosphine oxide (0.1 g), and 0.2 ml of oleic acid were placed into a 50-ml tri-neck flask, and heated to 180 °C. A solution of  $\text{Co}_2(\text{CO})_8$  (0.65 g in 3 ml of O-dichlorobenzene) was added by injection into the heated solution. After this addition, the reaction temperature was maintained at 180 °C for an hour. The reaction solution was then cooled to room temperature. Co nanoparticles of about 12 nm in diameter were produced in a yield of 95%.

(b) Preparation of Co/gold core/shell nanoparticles

The following is a typical coating protocol for Co/gold core/shell nanoparticles.

After Co nanoparticles (0.01  $\mu\text{mol}$ ) were dissolved in O-dichlorobenzene (12 g) in a 50-ml tri-neck flask, trioctylphosphine oxide (0.1 g) was added in the Co solution. The solution was heated to 180 °C, at which point the gold-shell stock solutions **1** and **2** (see below) were added dropwise, simultaneously, to the hot reaction solution, at a rate of about 50  $\mu\text{l}$  - 500  $\mu\text{l}/\text{min}$ . After sufficient amount of stock solutions **1** and **2** were added (about 5% excess), the reaction solution was maintained at 180 °C for another 30 mins. Subsequently, the reaction was cooled to room temperature in order to halt it.

The gold shell stock solutions were prepared as follows: stock solution **1**,  $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$  (0.1 g) and n-hexadecyltrimetyl ammonium bromide (0.1g) were dissolved in

O-dichlorobenzene (10 g); stock solution 2, 1,1-hexadecanediol (0.12g) was dissolved in O-dichlorobenzene (10 g).

5 The above examples merely serve to illustrate certain embodiments of the present invention and do not serve to limit it in its scope or spirit.

## REFERENCES

1. Mirkin, C. A.; Letsinger, R. L.; Mucic, R. C.; Storhoff, J. J. *Nature* **1996**, 382, 607.
2. (a) Elghanian, R.; Storhoff, J. J.; Mucic, R. C.; Letsinger, R. L.; Mirkin, C. A. *Science* **1997**, 277, 1078. (b) Taton, T. A.; Letsinger, R. L.; Mirkin, C. A. *Science* **1999**, 289, 1757. (c) Taton, T. A.; Lu, G.; Mirkin, C. A. *J. Am. Chem. Soc.* **2001**, 123, 5164. (d) Storhoff, J. J.; Elghanian, R.; Mucic, R. C.; Mirkin, C. A.; Letsinger, R. L. *J. Am. Chem. Soc.* **1998** 120, 1959.
3. (a) Mann, S.; Shenton, W.; Li, M.; Connolly, S. Fitzmaurice, D. *Adv. Mater.* **2000**, 12, 147. (b) Niemeyer, C. M.; Burger, W.; Peplies, J. *Angew. Chem., Int. Ed.* **2000**, 37, 2265.
4. Alivisatos, A. P.; Johnsson, K. P.; Peng, X.; Wilson, T. E.; Loweth C. J.; Bruchez, M. P., Jr.; Schultz, P.G. *Nature* **1996**, 382, 609.
5. Mitchell, G. P.; Mirkin, C. A.; Letsinger R. L. *J. Am. Chem. Soc.* **1999**, 121, 8122.
6. Chan, W. C. W.; Nie, S. *Science* **1998**, 281, 2016.
7. Mattoussi, H.; Mauro, J. M.; Goldman, E. R.; Anderson, G. P. Sundar V. C.; Mikulec F. V., Bawendi, M. G. *J. Am. Chem. Soc.* **2000**, 122, 12142.
8. He, L.; Musick, D. M.; Nicewarner, S. R.; Ssalinas, F. G.; Benkovic, S. J. Natan, M. J.; Keating, C. D. *J. Am. Chem. Soc.* **2000**, 122, 9071.
9. Pathak, S.; Choi, S. K.; Arnheim, N.; Thompson, M. E. *J. Am. Chem. Soc.* **2001**, 123, 4103.
10. Martin, B. R.; Dermody, D. J.; Reiss, B. D.; Fang, M.; Lyon, L. A.; Natan, M. J.; Mallouk, T. E. *Adv. Mater.* **1999**, 11, 1021.
11. Mulvaney, P. *Langmuir* **1996**, 12, 788.

12. Link, S.; Wang, Z. L.; El-Sayed, M. A. *J. Phys. Chem. B* **1999**, *103*, 3529.
13. Ung, T.; Liz-Marzan, L. M.; Mulvaney, P. *Langmuir* **1998**, *14*, 3740.
- 5
14. Lide, D. R. Eds, *Handbook of Chemistry and Physics*, CRC press: Boca Raton, 1992.
15. (a) Mulvaney, P.; Giersig, M.; Henglein, A. *J. Phys. Chem.* **1993**, *97*, 7061. (b) Rivas, L.; Sanchez-Cortes, S.; Garcia-Ramos, J. V.; Morcillo, G. *Langmuir* **2000**, *16*, 9722. (c)
- 10 **Ygeurabide US Patent 6,214,560.**
16. Schrock. E.; duManoir, S.; Veldman, T.; Schoell. B.; Wienberg, J.; FergusonSmith, M. A.; Ning, Y.; Ledbetter, D. H.; BarAm, I.; Soenksen, D.; Garini, Y.; Ried, T. *Science*, **1996**, *273*, 494.
- 15
17. C. Abdelghani-Jacquín et al., *Langmuir* **2001**, *17*, 2129-2136.